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TOWARDS COMPLEX ABIOTIC SYSTEMS FOR CHEMICAL AND BIOLOGICAL SENSING

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There have been at least two deeades of researeh foeused on developing biosensors, whieh mimie human physiologieal responses, but most of the work has attempted to eouple living eells, their reeeptors, and even seaffolded tissues to optieal or eleetronie sensors. All these biosensors had limitations imposed upon them by living systems. They required extensive life support and had very limited shelf life. Advanees in synthetie biology, materials seienee, moleeular engineering, and information teehnology have demonstrated the potential to ereate eompletely synthetie "abiotie" systems, whieh ean be programmed to possess the sense, response, and adaptation eapabilities of living systems. This report reviews the state of the art and suggests an approach to the design of abiotie systems for ehemieal and biologieal defense.

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PREFACE

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TOWARDS COMPLEX ABIOTIC SYSTEMS FOR CHEMICAL AND BIOLOGICAL SENSING

1. INTRODUCTION

The most obvious quality of living cells is that they are complicated and highly organized. However, these complex structures and functions are made from simple lifeless molecules, which, in appropriate combinations and numbers, can now be hypothetically put together in such a manner as to render 'lifelike' functional circuits and networks exhibiting distinct and extraordinary properties.

The most sensitive biodetector known today is the olfactory system of animal species, such as dogs and pigs,[1] capable of detecting a few molecules of C4 escaping from explosives hidden underwater, cocaine frozen inside ice blocks, or truffles buried underground. It was clear more than two decades ago that biological detection systems, which capitalize on an understanding of the molecular physiology of ligand-receptor interaction and the integrative properties of neuronal systems, offer the best prospects for developing true biosensors. An early 1980s concept for threat agent detection involved coupling neuroreceptors with microsensors to create biosensors capable of detecting classes of threat agents. For example, using the acetylcholine receptor as the biological recognition element (BRE) would enable the detection of more than 60 cholinergic neurotoxins, while using the enzyme acetylcholinesterase would permit detection of all organophosphorus nerve agents and pesticides.

Such broad spectrum, almost generic, biosensors are desirable for a number of reasons. From a military point of view, threat agents can be broadly considered to be xenobiotic entities, which interact with human physiology and in some way interrupt normal function. This interaction is mediated by BREs known generically as "receptors". Receptors are the key to communication among the body's many networks as well as with the external environment. Recently, a vast body of literature has identified nodes at the organ, cellular, sub-cellular, and genomic levels, which are critical to the proper function of these networks.

The nature of the military and, by extension, public health-threat is now such that it cannot be predicted *à priori*. Broadly defined, non-traditional agents (NTAs) include genetically engineered pathogens, mid-spectrum agents such as toxins and biological regulators, lentiviral and other vectors capable of introducing genetic material into host genomes, and new classes of chemical agents, to name but a few. The Cold War era approach of threat-oriented detection must necessarily give way to a target-oriented approach, the targets being BREs. While this shift was postulated two decades ago, the technology to bring it to fruition simply did not exist.

The technological bottlenecks in the 1980s were many. A number of receptors had been purified and studied but most were membrane bound and could not function outside of their native membranes, which were necessary to maintain proper conformation. Some receptors were cloned and produced in small quantities but manufacturing technology was essentially non-existent. Many studies, therefore, relied on isolating acetylcholine receptors from rich sources

such as the *torpedo* electric organ, or isolating synaptosomes from animal brains. Many receptors have since been cloned and efficiently expressed. G-protein-coupled receptors (GPCRs) are now known to be important targets for drug discovery and assay kits, for high throughput screening, have been developed.[2]

A second technological barrier was lack of knowledge about the interactions between BREs and non-biological supports. Specifically, techniques required to keep BREs and whole cells stable and functional on sensor platforms were very crude. Driven by the development of prosthetics and artificial organ systems, the interface between synthetic and biological materials has been an object of intense study in recent years. The National Science Foundation has established a Center for Biosurfaces at the State University of New York (Buffalo, NY).

Third, there was a limited ability to design novel BREs tailored for specific affinities or selectivity, nor was there much success with artificial receptors such as molecular imprints or engineered antibody fragments such as Fabs. Defining the minimum requirements for biomolecular interaction is critical for the design of stable artificial BREs. Two such approaches are the minimization of known protein binding motifs or, conversely, a biomimetic approach in which binding pockets are designed *de novo*.[3] Engineering novel antibodies is another successful approach, and these include recombinant antibody fragments (e.g., Fab, scFv) and variants such as diabodies and triabodies.[4] Finally, molecular imprints made from synthetic materials have been successfully applied to both analyte detection and drug release.[5] More importantly, the genomic tools required to identify all proteins needed for both normal and abnormal functioning, the precise role of the proteins in key metabolic pathways, or even determining a receptor's natural ligand did not exist. Using modern genomics mapping techniques, one can now identify all relevant proteins, but it is still problematic to definitively elucidate function. Nevertheless, enormous strides have been made in understanding GPCR cascades, signal amplification, and translocation of cellular proteins to specific intracellular compartments, knowledge which will result in the design of high content and throughput screens for drug discovery.[6]

Finally, the materials and micromachining techniques required to assemble an integrated biosensor were inadequate. Nanotechnology and its offshoots provide unprecedented control over material properties via structural control using directed- and self-assembly. Advances in microcontact printing, soft photolithography, microfluidics, and laser desorption techniques have proceeded rapidly in recent years. Micropatterning techniques, which include laminar flow patterning and microchannels, have resulted in the design of numerous functional biosensors.[7]

It is clear that a biomimicry approach in which one designs an artificial organism that possesses the functional equivalents of all of the key receptors and metabolic machinery required to detect and respond to toxicants in the external environment would address the needs of biological and chemical detection for military, medical, environmental, and industrial process control applications. It is also clear that advances in receptor biochemistry, synthetic and systems biology, metabolic engineering, bioinformatics, protein-protein and protein-surface interactions, and micro-manufacturing technologies have reached a point where it would be

possible to design a completely synthetic and intelligent sense and respond adaptive system. We propose such an Abiotic Networked Threat System (ANTS).

2. SYNTHETIC BIOLOGY

The term "synthetic biology" was coined in literature over 20 years ago. The first use of the term referred to the genetic engineering of bacteria using recombinant DNA technology[8] in which natural biological systems are altered by scientists to produce a biological system that performs unnatural function(s) – an approach that initiated the emerging field of 'bioengineering". The term "synthetic biology" was somewhat usurped in 2000[9] to describe the field of chemistry striving to design the synthesis of unnatural molecules that could function in living systems. In this use, synthetic biology became an extension of biomimetic chemistry, in which artificial molecules that can functionally replace natural biological molecules, such as artificial enzymes, aptamers, and peptide mimics are synthesized.[10,11,12,13] The extension of biomimetic chemistry to fulfill the larger scope of synthetic biology requires the assembly of individual chemical constructs into a larger chemical system that displays the natural functional properties of a living system such as sustained metabolic activity, cell synthesis, and genetic evolution. Most recently, the term synthetic biology has been adopted by an engineering cohort to define the process by which natural biological molecules (enzymes, DNA, proteins, etc.) are extracted from living systems and defined as basic building blocks to be reassembled in unnatural order and environments to create novel "devices or machines" that perform specific, predictable functions, which may or may not be found in natural biological systems.[9] This engineering approach differs significantly from "systems biology" in that the individual biological constructs most suited to constructing a device are those units that act independently in contributing to the whole: the whole can be predicted from the sum of its individual parts.

2.1 Artificial Cells

The unit of life is the cell, the smallest entity capable of displaying attributes associated with the living state: growth, metabolism, stimulus response, and replication. Even the simplest of cells, e.g., prokaryotes, possess and utilize highly integrated networks of chemical reactions. Despite great diversity in form and function, all cells share extensive biochemistry. How many genes does it take to make a cell or, beyond that, a network of different cells? Important insight can be gleaned from the smallest known genome for an independently replicating organism like *Mycoplasma genitalium* whose DNA consists of 517 genes in contrast to 30,000 genes in the human genome. Thus, there exists a broad hierarchy of cell complexity as well as component processes to borrow and to engineer into artificial systems.

A number of advances in fields such as evolutionary and synthetic biology and nanotechnology have made it possible to contemplate the development of acellular networks of synthetic genetic circuits (Xcells). In conception, Xcells would be non-replicating compartments genetically programmed to process information, make decisions, and carry out reactions, and would network with one another to form artificial ecologies (Xcologies) that would in turn perform higher order parallel and even emergent tasks.

Some of the advances that make possible Xcell and Xcology development include the explosion of knowledge about systems and synthetic biology, and the robust development of acellular biotechnologies. Furthermore, there is much precedent for formation of acellular supramolecular complexes of nucleic acid, either DNA or RNA, encapsulated in a protein coat and, in some instances, surrounded by a membrane envelope, i.e., the viruses. Although viruses are not alive, they represent supramolecular assemblies that act as parasites of cells, underscoring the functional 'culling out' of specific cellular processes albeit within the confines of living cells.

Systems biology is the integration of the many different levels of knowledge (genomics, proteomics, metabolomics) about cells and organisms to gain a global understanding of function. The informatics tools for systems biology, including databases such as Genbank and the Kyoto Encyclopedia of Genes and Genomes and analytical tools such as Cytoscape, now make possible the ready visualization and analysis of virtually any set of pathways, natural or contrived.

As a counterpart, genes, proteins, and functionalities are becoming increasingly fungible real-world entities that are being engineered as synthetic biology 'parts,' such as BioBricks™ (Cambridge, MA), that can be readily put together in novel synthetic genetic circuits. The modularity of synthetic biology parts is useful not only for creating circuits *in vivo* but also for generating tools and pathways in acellular systems, as well. The modularity of many BREs has become increasingly apparent from basic research into signal transduction and biotechnology explorations of directed evolution. Many cellular receptors and signal transduction enzymes can already be readily 're-wired' to take on new functions. For example, Wendell Lim and co-workers have re-tasked the yeast mating pathway to generate osmoresistance, as opposed to mating type changes.[14] New receptors can be created almost at will by directed evolution techniques such as phage display, cell surface display, and Systematic Evolution of Ligands by Exponential Enrichment (SELEX).

Other processes necessary to support BREs and signal transduction pathways can now be brought together in an acellular milieu. As a result of the years of product development in molecular biology, complex biological reactions such as transcription and translation can now routinely be carried out efficiently *in vitro*. Moreover, *in vitro* transcription, translation, and other cellular machines can be ensconced within *in vitro* compartments that resemble cells.[15,16]

Although it may be possible to standardize synthetic circuits in the context of a minimal biological cell, another, more visionary approach is to generate a cell-like compartment that could be reproduced and programmed to support synthetic circuits (Xcells). Elements that might be considered in Xcell approaches include the following aspects:

1. Beyond the *in vitro* compartmentalization methods so far cited, it may be possible to design and evolve self-amplifying or self-selecting genetic circuits completely within an Xcell[17] and to harness these to robust extracellular energy generating and harvesting systems for biology that have become available as a result of years of bioprocess development in industry.

2. The boundaries of the Xcell can potentially be much more robust and functional than conventional membrane-based vesicles or liposomes due to intense efforts in nanotechnology and drug delivery that have led to the generation of 'smart' materials and particles that are interactive with their environments. For example, it has now been proven possible to create 'double emulsions' in which dispersed water droplets are further compartmentalized and moved to aqueous environments, much as the bilayer allows cells to have an aqueous interior and exterior.[18] Moreover, by using surfactants with 'tails' that can be photopolymerized, it should be possible to encapsulate individual droplets into hard shells and actually make 'sheets' or films of droplets, much like colonies on a Petri plate. Alternatively, organotypic matrices such as hydrogel lattices or nanoporous materials might be used for the encapsulation or immobilization of genetic circuits.

3. Finally, efforts have led to methods for chemical, photoluminescent, and electronic signaling between small, independent biological or physical units. Transduction between biological recognition elements, signal transduction pathways, and signaling modalities sets the stage for the abiological networking of Xcells into Xcologies. Networked Xcells could evolve adaptive and emergent behaviors, similar in character to either molecular 'predator-prey' systems[19] or to cellular automata.

The development of an Xcell will involve integration of all of these disparate advances: an Xcell must be an encapsulated compartment in which chemical or light energy is captured and used for biopolymer synthesis. The biopolymers must be capable of processing and sending signals and performing programmed tasks based on communication with their environment. Communication between Xcells should lead to the competitive development of Xcologies that can perform a particular task or task set.

Beyond the technical achievements that will emerge from this program, there are fundamental scientific advantages to positing the development of Xcells and Xcologies, not the least of which is that it will better illuminate fundamental biological principles of living systems. Currently, the only example of living systems is cell-based, and there is no agreed upon or even self-consistent definition of 'life' or its properties. By providing an example of a programmable, acellular ecology, this largely philosophical problem is deftly avoided and engineers can begin to understand what principles and practices are most suited to adapting living systems and their mimics to military tasks. Beyond this advantage, having one example of a programmable Xcell will allow engineering standards to finally be set in place for further development. While explorations of synthetic biology have not so far yielded anything equivalent to VLSI, it seems likely that the production of Xcell circuitry will engender a revolution similar to the one that occurred in electronics in the early 80s.

2.2 Natural Proteins as Engineering Units

Proteins exhibit a wide diversity of physical and catalytic properties. The question of whether enzymes involved in known metabolic pathways can be isolated and reassembled as basic building blocks to generate new pathways has been pursued for several decades. Recombinant DNA technology has significantly aided the ability to pick and choose enzymes from various organisms and then direct their reassembly into a single organism, hence conferring the ability to synthesize an unnatural product in the host organism.[20] Here, bacterial strains are genetically engineered by inserting genes for various synthetic steps in the metabolic pathways from several different microorganisms into the host. A strain of *Escherichia coli*, for example, has been developed that can synthesize an isoprenoid precursor for the antimalarial drug artemisinin using enzymes assembled from three different microorganisms.[21] Genes from two other organisms were introduced into the *Ralstonia eutropha* bacterium to produce a co-polymer from fructose.[22]

2.3 Genetic Regulation and Signaling Pathways

A prime example of modularity in biological systems is evident in genetic regulatory and signaling pathways, where combinations of proteins can function as molecular switches affecting gene expression and ultimately the phenotype. Thus, the "wiring" of the many signal transduction pathways is based upon particular protein domains present in transduction proteins that bring components of signal transduction pathways together. Here, ligand binding, chemical reactions, or the physical translocation of a component generates an event which can serve as an input for another switch.[23,24] Using this approach, synthetic circuits have been synthesized that show AND and OR logic gating based on ligand input.[25,26,27] An artificial oscillatory network has also been constructed from three transcriptional repressor systems that are not naturally paired in any biological system,[28] and a molecular automaton that plays tic-tac-toe against a human has been designed based on 23 molecular scale logic gates using deoxyribozymes as the building module.[29]

Signal-transduction pathways have many common functional/architectural elements and hierarchical structures enabling the creation of "plug & play" signal transduction cassettes that can be incorporated into acellular systems. Due to their simplicity, microbes represent a source from which much can be borrowed for purposes of engineering acellular networks. Microbial quorum sensing is responsible for a variety of phenotypes and is rich in diversity, and modes of action. As such, quorum sensing represents a "guide" for learning how signals can be translated to altered phenotype(s) as well as for the designing of useful acellular prototype signal transduction networks. Quorum sensing may be the foundation upon which the more sophisticated intracellular communication found in higher order organisms has evolved. If this is the case, methods that incorporate native signaling architecture may be engineered for acellular network purposes, exerting greater control with less collateral damage to other resident, non-targeted processes.

Although there have been few technological or commercial applications derived directly from adapting or rewiring the quorum sensing signaling process, metabolically engineered signaling modules have been constructed to alter phenotype giving rise to

recombinant gene products.[30,31] For example, Bulter *et al.*[32] created an artificial genetic switch using acetate for modulating cell to cell signaling in *E. coli*. Neddermann *et al.*[33] developed a hybrid expression system by incorporating the quorum circuitry of *Agrobaterium tumefaciens* into a eukaryotic transcriptional controller for HeLa cells. Weber *et al.*[34] implanted the *Streptomyces* bacterial QS system for tuning heterologous protein expression in mammalian cell culture and mice (human primary and mouse embryonic stem cells).

The molecular basis by which quorum sensing works varies among different species, both in the synthesis of the signaling compounds and in the modes of their perception. However, this diversity affords a vast array of potential acellular applications–the types of rewired systems for achieving specific outcomes can vary greatly and are limited by 1) imagination and 2) ability to abstract regulatory features and reconstruct them into modular, transplantable, controllers. There are also many modes by which the regulatory function is translated into altered gene expression. Most of the well-studied systems modulate protein transcriptional regulators. Additionally, there is also evidence that small RNAs are involved as riboregulators and post-transcriptional regulation has been linked to quorum sensing systems and quorum sensing signal transduction.

2.4 Devices

A device is construed from the decomposition of a system into basic functional parts that perform physical processes. Devices process inputs to produce outputs by performing metabolic or biosynthetic functions and interface with other devices and the environment. Devices can represent one or more biochemical reactions involving transcription, translation, ligand/receptor binding, protein phosphorylation, or enzymatic reactions. Devices can be composed of few reactants (e.g., a protein phosphorylation device consists of a kinase and substrate), or multiple reactants and products (e.g., a regulated gene, transcription factors, promoter site and RNA polymerase compose a transcriptional device). Devices can be extracted unaltered from nature, such as transcription factors and promoter of a regulated gene, or by modifying biochemical reactions. Transcription or translation control devices are controlled by the specificity and efficiency of nucleotide interactions and are relatively easy to build based on the specified order of the reaction. Transcriptional control systems have been employed for signal amplification, control of downstream devices, and multifactorial control by multiple transcription factors.[35,36,37] While transcription and translational devices are fairly easy to connect and can offer great logical complexity, changes in output are relatively slow (minutes) and consume a large amount of cellular resources to support the protein synthesis events that mediate the output.

Devices employing protein-ligand or protein-protein interactions have different input-output characteristics and usually require more complex modification of their natural substrates. Protein switches have been created by inserting allosteric domains into existing enzymes, replacing protein domains randomly followed by directed evolution, or using computational chemistry to model receptor binding sites and selectively mutate key residues to confer altered activity. Multiple examples exist of modifying proteins/enzymes to significantly alter their ligand/substrate response and serve as the initial input device for extended metabolic cascades.[38,39,40,41,42] Designing and building protein interaction devices carries several caveats.

The proteins must be well characterized to allow the designer to determine where deletions, additions, or replacements of key domains should or can occur so as not to negate the protein's 3D structure that plays a critical role in protein interactions.

Connecting protein devices also poses more difficulty than connecting translation/ transcriptional devices. Protein devices must be well matched and one must validate that information transfer between devices is predictable and reliable. The benefits of protein interaction devices are that output is very fast (output in sub-second timescale), signals can be readily amplified by other cell reactions (i.e., kinases and enzymes) and proteins can mediate repeated interactions while consuming nominal cellular resources.

2.5 Modules

A module is defined as a distinct set of individual devices with well-defined interconnections and functions that can perform a more complex task than a device. In the natural cell, modules are defined in the form of pathways, such as a metabolic or signal transduction pathway. Synthetic biology seeks to understand and establish the rules of composition whereby the function of a whole pathway can be derived from the additive function of each component part, which can be engineered as a device. Constructing modules from wild-type devices found in naturally occurring systems is rarely successful since evolution has already selected wild-type devices to perform optimally in their natural context. Modeling and rational mutation of devices has proven effective in altering wild-type devices to properly interact with each other when combined into a module function, as long as the properties of the individual devices are well known. In the absence of well known characteristics of a device, where rational mutation of selected sites would be expected to yield improved properties, directed evolution has proven useful in optimizing device and module functionality. The onus of directed evolution is having some *à priori* idea of what features of the system need to evolve and being able to apply selective pressure toward those criteria.

Synthetic transcriptional regulatory networks are the best characterized modules created to date and include cascade, feed-forward, and feedback motifs. Cascade modules generally generate a steady-state output that is monotonic to its input, though physical variations have shown their ability to control temporal sequencing of gene expression and the ability to attenuate gene expression noise. Two feed-forward transcription modules have been constructed and allow for a transient non-monotonic response to a step-like stimulus. Incoherent feed-forward modules accelerate response to the step-up in stimulus but not the step down. Coherent feed-forward modules delay response to the step up in stimulus, but not the step down. These constructs have yielded modules that can function as persistence detectors, delay elements and pulse generators which conceivably could be used to construct parallel circuits to carry information at different rates.[43,44,45] Applying regulatory feedback to a module can provide state and memory functions. Positive feedback produces bistability while negative feedback can yield oscillation. Genetic "toggle" switches and various oscillators have been produced using regulatory feedback designs.[47,47,48]

Synthetic protein signaling modules have focused on modifying and assembling signal transduction devices. Synthetic metabolic networks use transcriptional and translational control elements to regulate the expression of enzymes that synthesize or break down

metabolites. Here, metabolite concentration serves as an input for other devices. In the most publicized demonstration of this approach, Martin et al. transplanted the entire mevalonate isoprenoid pathway for synthesizing isopentyl pyrophosphate from *Saccharomyces cerevisiae* into *E. coli*, which, when coupled with an inserted synthetic amorpha-4, 11-diene synthase, produced the precursor to the antimalarial drug artemisinin. Artificial circuits have also been demonstrated where metabolic pathways have been connected to regulatory proteins and transcriptional control elements.[49]

Two major metrics of success in constructing functional devices and modules in synthetic biology are predictability and reliability, where the system performs the function for which it was designed at the expected level of fidelity each time. It is prudent to consider that functional modules will typically reside in a cell environment that is subject to a range of biological uncertainties and cell "noise" stemming from other cell functions/processes. Synthetic biology does not demand that cells behave perfectly, only that a significant number of the cell population performs the desired task. Design and fabrication methods that account for this uncertainty will greatly facilitate the on-demand engineering, versatility and robustness of biological devices and modules.

2.6 Synthetic Organelles

Nature provides exquisitely refined and accurate capabilities that are thus far impossible to recreate in abiotic structures, two of which are ultra high-resolution recognition and the assembly of multi-component enzymatic complexes. Mimicking such processes using abiotic structures will create, for the first time, a generic, bioinspired enzymatic technique that enables the template-directed synthesis of a biochemical pathway within a compartmentalized, abiotic, multi-component macromolecule. The targeted assembly of proteins is an essential aspect of life. Within a cell, protein assembly is often directed through the compartmentalization of individual proteins into specific organelles or membranes. Compartmentalization provides a method for concentrating and, therefore, enhancing the assembly of functional macromolecular structures such as the multi-component light harvesting structures found in chloroplasts or the transcriptional complexes located in the nucleus. In almost all cases, specific signal peptides direct this targeted compartmentalization within a cell. Signal peptides are generally short, 3 to 60 amino acids in length. Each class of signal peptide interacts with an organelle specific protein to facilitate its import and/or retention. Using this process as a model, it will be possible to use engineered signal peptides for the bioinspired compartmentalization of proteins within molecularly imprinted polymers.

Molecular imprinting is a technique that creates synthetic materials containing highly specific receptor sites having an affinity for a target molecule. Three-dimensional cavities are created within a polymeric matrix complementary to the size, shape, and functional group orientation of the target molecule. The size and shape of the cavity allow the target molecule or similar molecules to occupy the cavity space, while the functional group orientation within the cavity will preferentially bind in specific locations to only the target molecule and not to similar molecules. Molecular imprinted polymers (MIPs) can mimic the recognition and binding capabilities of natural biomolecules such as antibodies and enzymes. The MIPs have several

advantages over natural biomolecules, such as modular synthesis that can provide functional hydrogels in high-yields and selectivities.

The MIP hydrogel can be treated like a cellular organelle, which could be engineered to bind specific signal peptide sequences. Thus, in order to compartmentalize any set of proteins into the MIPs, one would need to attach a specific signal sequence to them in order to mimic enzymatic chemistry inside the imprinted hydrogel. This method, if successful, would have the benefit of being universal, (i.e.) any set of proteins could be assembled as long as they contain the right signal peptide. Thus, the potential to do multi-component enzymatic chemistry directly in the hydrogel would be considerably higher. Additionally, MIPs could be imprinted with multiple tags, perhaps even patterned in some way to create a desired network. By arranging the spatial configuration of enzymes in an MIP cavity, one may be able to alter product specificity, much like reengineering the active site in directed evolution studies. To accomplish this, it is necessary to make signal peptides of specific geometries that, when imprinted into a polymer hydrogel, can later be recognized for assembly of a multi-protein complex.

3. MATERIALS FOR MEMBRANES AND SUPPORT

One of the challenges to the design of abiotic sensors will be to select and develop appropriate technologies for the environmental interface, support structure and/or membranes within and through which the functional components work. Because the functional components are the heart of the system, the specifics of these supports need to be tailored to the structure and function of the components, yet the successful interactions of the components are likely to be at least partially dependant on the membranes and support materials. Thus, it is essential to take a holistic approach and address promising membranes and support technologies that could support any of a number of likely scenarios. This will provide the agility to construct an encapsulation and support system appropriate to the functional technologies as they develop and mature.

During the development of the functional components, there will be needs for testing and integrating well before technologies are fully downselected. Using an agile approach to materials technology will facilitate these evaluation steps and maximize the potential for each technology addressed in the program.

Historically one of the failing points of biotic sensors was the environmental needs associated with maintaining cell viability. In general, biochemical activity is carried out in aqueous solutions that make it incompatible with long-term unattended operation in real-life scenarios. Abiotic functional components may share some of these issues, depending on the fidelity with which the abiotic synthesis mimics natural biology. The matrix materials will need to address the range of environmental dependence and consider the possibility of hybrid matrices to address different levels of dependence within one sensor. This poses the following scenarios:

1. The functional components are subject to the same or equivalent environmental requirements as their biological homologues and require the same or equivalent through-membrane and intra-cellular mobility.

2. The functional components are not dependant on environment but do require through-membrane and intra-cellular mobility.

3. The functional components do not depend on the environment nor do they require through-membrane mobility, but they do require intra-cellular mobility.

4. The functional components do not depend on the environment nor do they depend on intra-cellular mobility, but they do require through-membrane mobility.

Again, historically biotic sensors were based on living cells or tissues and the morphology of these cells and tissues were fixed by nature. In contrast, abiotic morphologies will be driven by functional aspects of how the evolved system is to be deployed, operate (functional components), and communicate. The morphology issue will have some bearing on appropriate matrix material, while again the matrix material requirements of the functional components may partially dictate the available morphologies and deployment/communication options. This situation will also benefit from the holistic approach.

Recognition, transduction, and associated devices will be evolving concurrent with the evolution of an appropriate support matrix. The purpose of the support matrix research is two-fold: first, to provide appropriate materials in which to study evolving synthetic biology products and second, to work toward hardened materials that include both functionalized and support aspects, tailored to the abiotic cell product.

Research will begin by supporting BRE research at the current state-of-the-art. Initial goals will be to rapidly provide matrices in which early BREs and transduction devices can be studied. For these purposes, it will be necessary to build on existing technologies associated with channel based sensing, starting with lipid bilayers and suspended liposomes. Stabilization of these "conventional" membranes using nanoparticle technology will likely lead to the following:

1. Extend the viability of these membranes to allow temporally extended experiments on device function;

2. Elucidate fundamental facts concerning the distinction between lipid-suspended particles and lipid-wetted agglomerates, i.e., at what particle loading does a liposome lose its properties vis-à-vis BRE functionalization and how do these limits affect device function; and

3. Determine whether nanoparticles can function as rafts in a lipid bilayer system.

The results of this stage of research are unlikely to effect a hardened matrix for a final product; they will facilitate synthetic biology work toward the required devices and may provide essential parts of an inhomogeneous or layered "cell".

Beyond the fragile lipid-based matrices are supported membranes, hydrogels, and chitosan products. All of these approaches have been used in conjunction with synthetic biology

or related research and all provide some stability advantage over simple lipid membranes or liposomes. Hydrogels and supported membranes are likely to provide the "breadboards" for device development and integration while the unique properties of chitosan make it a likely candidate both for use in evolving breadboard systems and as a component in hardened cells.

Moving toward Xcells hardened against the environment and suitable for incorporation into myriad military systems, membrane and matrix support directions will need to be tailored to evolving functional components. At this stage, the rapid advances in directed- and self-assembly will lead to manufacturing tools, while materials including porous inorganics, polymerosomes shells, chitosan, cellulosic materials and aerogels will have advantageous properties. Structure analysis will be the key, along with functionalization and device interface. Hybridization of materials to provide appropriate mix of properties is likely to be required where assembly technology alone cannot provide the necessary functions in homogeneous materials.

3.1 Channel Based Sensing

Membrane proteins represent one-third of the human proteome[50] and play essential physiological functions: controlling interactions between cells and their environment, facilitating signaling, and helping to regulate the composition of the cytosol. The crucial physiological roles they play make them of central pharmaceutical importance; membrane proteins represent two-thirds of all drug targets[51] and are also screening targets for unintended interactions with all novel drugs.[52,53,54] The susceptibility of membrane proteins to pharmaceuticals is reflected in their vulnerability to chemical and biological warfare agents. The ricin B chain, for instance, binds to glycosalated membrane proteins to facilitate entry of the ricin toxin into the cell. Membrane channel proteins, which control the flow of ions into and out of cells, are the targets of all neurotoxins.

Channel proteins have also been the subject of intense recent research as 'stochastic sensors,' a new kind of protein-based sensor capable of chemical detection at the single-molecule level. Stochastic sensors are part of a general class of resistive pulse sensor such as a Coulter counter,[55] which is used to count cells and particles ranging from sub-micron to millimeters in size. Resistive pulse sensors measure the ionic currents in an electrolyte modulated as a result of particle flows through a small pore. The size and number of the particles can be inferred through the magnitude and frequency of the current blockages. As the pore is shrunk to the nanoscale, the scale of a channel protein, the "particles" it is capable of sensing are on the scale of small molecules. The frequency of the conductance blockages and their duration is dependent on the molecular concentration, the molecular affinity for the nanopore, and the transit time of the molecule through the pore. Since different molecules have different statistical signatures, it is possible to detect more than one sub-type of molecular species simultaneously with a single sensor through analysis of the transport record. The first and most in-depth work concerning stochastic sensors has been done using the channel-forming antibacterial protein α-hemolysin (αHL). This soluble protein spontaneously inserts itself into lipid bilayer membranes, forming a heptameric channel 2.9 nm in diameter at the exterior and narrowing to 1.5 nm in the interior, with a conductance of 0.8 nS.[56] αHL has been engineered to have sensory capability to specific drugs and organic ligands,[57] hybridization events of DNA[58] and second messengers,[58] 2,4,6-Trinitrotoluene (TNT),[59] and other organic molecules.[60] A key

advantage to protein-based stochastic sensors is that, with relatively minor modifications, the same protein and sensor platform can be used to detect a variety of molecules.

With the ability to rapidly identify and determine the quantity of various molecules in complex mixtures, practical stochastic sensing devices have the potential to be valuable diagnostic tools, rapidly measuring the quantities of small-molecule analytes of interest in urine, blood, or saliva samples. Their capacity for sensing drug molecules has direct applications to testing for illicit drugs and properly metering therapeutics with unpredictable pharmacokinetics or narrow therapeutic indices, such as warfarin and digoxin. The high speed and small size of these technologies makes them a natural fit for rapid diagnosis and testing at the point of care in a clinician's office or in a patient's home. These same characteristics also make stochastic sensing an ideal technology for environmental monitoring of all threat agents. The ability of stochastic sensors to detect small concentrations of arbitrary small molecules makes them potentially powerful tools for detecting chemical warfare agents.

Another nanopore resistive pulse sensing application is the sequence analysis of DNA and RNA as it is threaded through a nanopore. The first nanopore measurements utilized αHL to measure single-stranded RNA and DNA[61] of various compositions and lengths. The temporal resolution of the experiment did not permit the detection of single nucleotides traveling through the pore. Later work showed the ability to simultaneously distinguish between 125 base polyA and polyC homopolymers of RNA through examination of the amplitude of the current blockades, an important result showing the plausibility of differentiating individual A and C bases as they traverse the pore.[62] While these technologies have yet to accomplish the capacity to sequence DNA, they are moving rapidly towards this achievement. Aside from the obvious benefits of a rapid single-molecule DNA sequencing technology to basic research, such a technology also has applications in health care and environmental monitoring. Genetic screening and pharmacogenetic medicine require a means to rapidly determine a patient's genotype. Rapid sequencing will allow for fast detection and accurate identification of dangerous microbes, as well as the identification of threat pathogens in complex samples based on techniques from environmental genetics.

Although the preceding discussion has focused on sensing based on resistive pulses in ion channels, the same methods can be used to measure the behavior of physiologically important ion channels (e.g., K, Ca, Na, AChR channels) *in vitro*. By measuring any changes in conductance properties in the presence of potential threats, these channels can directly indicate danger to human health because a measured change in conductance measured *in vitro* directly indicates an adverse reaction *in vivo*. As such, sensors based on physiologically relevant proteins would respond immediately to any chemical with a neurotoxic effect, even one that has not been previously identified.

Measuring the ionic conductance through a channel protein requires the incorporation of the protein into ~5 nm thick lipid bilayer membranes. The membranes are electrically insulating and the only conduction path for ions is through the incorporated channel protein. The ionic current through an incorporated channel protein can be modulated by protein conformational changes (e.g., allosteric gating) or by the presence of analytes within the channel.

Despite the enormous potential of channel protein-based sensing, devices to perform or exploit these measurements are not practical for widespread use because they depend on the labor-intensive formation of fragile and subsequently short-lived lipid bilayer membranes.

3.2 Lipid Bilayer Techniques

A widely used tool for studying channel proteins is the so-called 'black lipid membrane' (BLM).[63] A non-polar solution containing lipids is "painted" across a tiny orifice in a wall between two chambers filled with aqueous solutions;[64] lipid molecules self-assemble tail-to-tail across the orifice to form a freestanding planar bilayer membrane. In such a configuration, the electrical properties of lipid bilayer membranes are interrogated by means of Ag/AgCl electrodes in each chamber. The membrane resistance may be measured directly (resistances greater than 1 GΩ indicate a high quality membrane) and the membrane thickness may be determined through a combination of optical observations to measure the membrane area and electrical measurements of the membrane capacitance.[65] When channel proteins are introduced to a chamber containing a freestanding lipid bilayer membrane, they can insert into the membrane,[64] an event indicated by a sudden jump in membrane conductance. Measurements of this conductance are necessary for membrane channel-based research and applications.

While such systems enable single-molecule observations of protein channels, they face practical shortcomings that limit their widespread application and restrain the development of high-throughput membrane channel screening and protein channel-based sensing. First, freestanding lipid bilayer membranes are difficult to fabricate: membrane reconstitution methods require significant investments in operator training and are not amenable to automation. Once successfully formed, these membranes are fragile, rupturing with slight vibration and requiring mechanical isolation. Finally, research with freestanding lipid membranes is limited by their short lifetime: they typically last in the laboratory for no longer than a day, placing a fundamental limitation on possible experiments and devices.

These issues are the primary obstacles to the practical application of sensors containing channel proteins. Practical molecular sensing technologies simply cannot accommodate the complex, fragile, and operator-intensive apparatus required to study ion channels in reconstituted lipid membranes. These applications require a membrane system that can deliver results quickly and automatically. Practical membrane protein-based sensor devices must be portable and autonomous to be useful outside the laboratory. However, existing membrane formation techniques are tied to the laboratory, requiring a skilled operator to form the membrane and a vibration isolation apparatus to maintain an intact membrane. Bringing these systems out of the laboratory requires a membrane technology to make them portable and long-lasting or to assemble them automatically at the point of use. The current techniques and technologies for creating freestanding planar lipid bilayer membranes in the laboratory have essentially remained unchanged over the past 30 years. Scientific studies, clinical research, and technological applications of channel proteins, but most importantly threat detection and identification, would all benefit greatly from a robust, long-lived, compact, and easily operated membrane platform.

3.3 Supported Membranes

The fragility of lipid bilayer membranes is a well-recognized problem. One popular approach to stabilizing lipid bilayers has been to fabricate these bilayers directly on a solid support substrate. The solid support can impart considerable stability to these bilayers: solid-supported membranes don't require mechanical isolation; they can remain intact for over a week,[66] and some supported membrane systems even remain stable in air.[67] However, the solid support introduces some new complications. For instance, inserting a transmembrane protein into a supported membrane can be difficult since such proteins protrude through the membrane and abut the solid support. This is especially limiting in the case of channel proteins, where the solid support can block ion transport through the protein.

To allow for the insertion of proteins in solid-supported membranes, several "tethered" membrane systems have been developed. These systems use a spacer molecule (either a macromolecule or an extended-conformation acryl chain) to put some distance between the solid surface and the membrane. This creates a thin electrolyte reservoir between the surface and the membrane into which membrane proteins can protrude, allowing for free protein insertion into the membrane and transport of ions between the electrolyte reservoir and the bulk solution exterior to the membrane. While this solves the problem of protein insertion, the limited volume of the electrolyte reservoir limits the duration of DC-driven ionic transport it is capable of measuring, since application of a DC voltage changes the ion concentration in the reservoir and leads to rapid changes in the measured conductance.[68] Freestanding membranes have effective infinite electrolyte reservoirs and no inherent limit on the duration of applied bias they are capable of sustaining.

The nature of the solid support leads to further complications in the measurement of ion channel conductance in supported membranes. Freestanding planar membranes are easily accessible to a variety of electrodes; silver-silver chloride electrodes, for instance, allow for the direct measurement of chloride, potassium, and sodium ion currents through lipid membranes. In supported systems, however, the material on which the membrane is fabricated must serve as an electrode. Supported membranes are typically fabricated on gold, silicon, or silicon dioxide surfaces. As electrode materials, these surfaces make the direct measurement of ion transport through the membrane impossible. Rather, indirect methods such as AC impedance spectroscopy must be used in which a frequency-domain analysis of the membrane's response to electrical stimulation is performed. These frequency domain data are then fit to analytic models or simulations of membrane behavior to obtain information on the electrical properties of membranes. Aside from being ponderous to implement, impedance spectroscopy has very coarse time resolution, limited in tradition techniques to tens of minutes.[69] Ion transport measurements for channel sensing require time resolution in the millisecond range, which can only be delivered by direct measurement of ion transport.

Researchers have also encountered difficulties in fabricating supported membranes with the high-quality gigaohm resistive seals required for single-molecule ion channel sensing. Since the size of supported and freestanding membranes can vary widely, and resistance is dependant on membrane area, it is easiest to compare the quality of membrane seals by comparing specific resistance, or resistance normalized over membrane area. The free-

standing membranes formed have specific resistances in the range of 20-100 MΩ cm^2. This compares to high-resistance tethered membranes with specific resistances in the range of 0.5-1 MΩ cm^2. While ion channel proteins have been electrically observed in solid-supported membranes, these proteins were analyzed as ensembles that decreased the total resistance of the membrane rather than as single molecules.[70,71] High resistance seals are necessary for the low-noise detection of the interaction of small molecules and ion channels that forms the basis of stochastic sensing. Fourier transform impedance spectroscopy technologies have the potential to improve time resolution,[72] and field-effect transistor-based sensing based on semiconductor supports could allow for more direct current measurement.[73] Efforts to improve membrane resistance via miniaturization and investigation of novel materials are also ongoing.[74]

3.4 Hydrogels

Hydrogels are appealing alternative materials for membrane supports. They can provide mechanical stability while allowing the membrane access to a bulk-like aqueous environment, enabling a low-resistance path to the membrane-incorporated protein for ionic currents and diffusing analytes. Hydrogels have been used previously to support membranes. In these cases, lipid solutions were deposited on top of pre-cast gels, but the quality of the resultant membranes was too poor for a single channel measurement.[75,76,77] Ide and Yanagida formed high resistance free-standing membranes and subsequently brought them into contact with a pre-cast gel on one side.[78,79] Although single channels were measured in that work, the lifetime and fragility of the membranes was unimproved over conventional membranes. Peterson and coworkers physically sandwiched a lipid membrane between two pre-formed slabs of gel.[80,81] However, this did not show sufficiently high membrane resistances to enable single-molecule measurements. All of these efforts have relied on pre-cast gels, and they have met with limited success. Jeon et al. have developed an *in situ* encapsulation system in which a hydrogel is photo-polymerized around a conventionally formed bilayer membrane, resulting in robust, long-lived, high-quality membranes.[82] These membranes—together with incorporated channel proteins—easily withstand mechanical perturbations that would immediately rupture conventional membranes.

3.5 Chitosan

Individually, biotechnology and microelectronics revolutionized our ability to detect and communicate threats. Biotechnology provides the tailored biological components to recognize threats to specific physiological targets. Microelectronics provides autonomous devices that are cheap, robust, and small and offer data and signal processing capabilities for near-instantaneous communication over vast distances. The convergence of biotechnology and microelectronics will allow unprecedented capabilities for recognizing and responding to a plethora of threats, but this coupling will require the seamless integration of biological components into microelectronic devices.

Biology and microelectronics communicate through markedly different signaling modes. Electronic devices signal through the flow of electrons or the transmission of light, while biology signals with ions (e.g., Na^+, K^+, and Ca^{2+}) and molecules (e.g., hormones and

neurotransmitters). Bridging this communication gap is essential to fusing the individual strengths of biology and electronics.

Chitosan possesses unique properties that enable it to interconnect biology and devices.[83] Chitosan can be "connected" to device addresses because the pH-responsive film-forming properties of this aminopolysaccharide allow electrodeposition in response to localized electrical signals.[84,85] Chitosan's electrodeposition is spatially[86] and temporally[87] selective, while the electrodeposited films are stable in the absence of an applied voltage. Chitosan can be "connccted" to bio-components (e.g., nucleic acids,[88] proteins,[89,90,91] and virus particles[92]) in two different ways - chitosan's nucleophilicity facilitates covalent conjugation,[93] while the electrodeposited film can entrap nano-scale components[94] (e.g., proteins). Thus, chitosan allows device-imposed electrical signals to guide the interconnection of biological components to devices.[95]

In addition to enabling bio-device interconnection, chitosan possesses properties that allow biological signals to be transduced into signals readily processed by common devices. Specifically, chitosan films are permeable to ions and small molecules allowing signaling through electrical modes.[96,97] Further, chitosan films are transparent which facilitates signaling through optical modes (e.g., through integrated waveguides).[98] Finally, chitosan films are stimuli-responsive enabling mechanotransduction.[99] Thus, chitosan can transduce environmental stimuli into multiple signaling modes.

4. POWER

Biological systems operate on energy transductions in the mitochondria. That is, the energy powering the cells is derived biochemically. In the case of an abiotic system performing selected cell-like functions and modeled almost exclusively on biotic cell architecture, the biochemical approach will provide the most directly usable form of energy. In order to duplicate the energy-producing biochemical functions in the abiotic cell, one must ensure the following:

1. Determine energy requirements of the abiotic system,
2. Develop chemical reactions that are triggered by recognition events, and
3. Couple that energy to the signaling event.

These small scale energy requirements differ markedly from traditional power and energy issues faced by macroscopic and even mesoscopic equipment.

On thc small scales associated with cell function, whether artificial or biotic, it is possible to consider energy on the basis of thermodynamics of individual molecular interactions. In the simplest of terms, some will require energy and some will release energy. In a living eukaryotic cell, ATP is synthesized from ADP and P_i and oxidative phosphorylation involving input energy derived from dissipation of a proton gradient or electrical potential. However, this

is not the only source of ATP generation.

Phosphorylation of ADP to ATP is also observed coupled to the following:

1. Oxidation via thioester intermediate formation, and

2. Dehydration reactions resulting in generation of enol phosphates that have high phosphoryl transfer potential as typified by the two ATP generating steps in Glycolysis.

It is noteworthy that the above two reactions represent the sole source of *de novo* synthesis of ATP in the red blood cell. Thus, engineering of circuits involving the respective glycolytic enzymes which are cytosolic (soluble) within a compartment could serve as a source of ATP generation for other ATP requiring circuits as long as a few key metabolites (3-Phosphoglyceraldehyde, NAD^+, Pi, and pyruvate) were provided the system. A lactate dehydrogenase 'recycling' circuit can even be included to recycle reduced NAD^+, i.e., NADH as well as pH control. Therefore, acellular 'substrate level phosphorylation' circuits are well within reach due to the soluble nature of this system and its not requiring elaborate membrane bound oxidative phosphorylation functions, i.e., electron transport. Thus, in an abiotic system adopting cell-like functions, the energy required to drive these functions can be engineered to include such functions and capabilities.

Substrate phosphorylation, alluded to above, constitutes a recognition event resulting in a thermodynamically spontaneous reaction that can supply enough power for subsequent signal transduction events. Additionally, an external energy source can be coupled to a catalytic wheel and provide the activation energy for ion pumping.[100] Understanding biological energy balances and duplicating them in abiotic systems is a promising approach to powering the cellular functions mimicked in an abiotic cell. The goal in the case of an abiotic cell is to use an elegant thermodynamic design as well as the partitioning of the reactions involved in recognition, signaling, and transduction, taking advantage of catalytic enzyme activity, substrate binding, and conformational events.

Although ideally, the cells would 'find', convert, and use energy on an as-needed basis, the practical use of power storage requirements need to be considered. Nanocapacitors[101] have been demonstrated to be practical approaches to this problem and have in fact been used on the meso scale to power telemetry functions. In separate investigations, carbon nanotubes have been used to enhance the efficiency of ultracapacitors. The possibility exists through combined technologies to develop a nano-enhanced nano-ultracapacitor to store and supply energy converted from environmental, solar, or thermal energy.

In a living system, interactions between the organism and the environment typically involve cooperative functioning of many cells. In the special case of single cell organisms, these interactions are either limited or dependent on the multiplicity of organisms, i.e., bacteria respond to their environment by multiplying and affect their surroundings by the cooperative effects of many cells.

In the case of abiotic unicellular systems, it is likely that the energy requirements for signal amplification and communication to macroscopic indicators or telemetry will not be served by simulated biochemical energy. It is also an undeniable fact that biological systems, efficient though they are, do require a certain amount of "input" fuel and it may not be possible to load an abiotic cell with sufficient fuel to support functioning throughout its lifetime. For these situations, alternate energy sources must be considered.

With the expanding volume of research in nanotechnology and microelectro-mechanical systems (MEMS), there have been significant strides in the areas of nanocapacitors, nanowires, nontraditional solar photovoltaics, thermal photovoltaics, and biomimetic solar energy conversion. These, combined with conductive polymer technology and recent successes in the area of molecular switching, provide ample technology for powering and controlling signaling and telemetry operations in an abiotic cell. For these requirements of tailored application of nontraditional but universally applicable power sources, we will rely on the research and technology advances of existing energy and power programs.

The level of amplification of the desired signal and the process of telemetry are not native functions of typical biotic cells and it will be necessary to link the cell signal to a non-native cell function. This may be achieved by the use of nanowires and nanoswitches. The use of nanowires to detect events in single cells has been demonstrated for the case of live neuron activity and photon activity.[102,103] Biological molecules that operate as molecular switches have been identified and the switching mechanisms have been demonstrated up to and including the complexity of a 4-way switch.[104]

Harnessing of ubiquitous environmental energy to power amplification and telemetry will be essential to a self-maintaining system. The two most amenable sources of power for these applications are solar and thermal, and the energy conversion processes of photosynthesis can be mimicked abiotically.[105] Like the mitochondrial based oxidative phosphorylation systems, photosynthetic systems also utilize a proton gradient in oxygenic photosynthesis. There are numerous photosynthetic bacterial systems from which abiotic circuit components can be modeled. Solar photovoltaics based on nanoparticles have been demonstrated at the macro scale and, in fact, are mature to the level of commercialization. Engineering these materials to a smaller scale has not been reported although this is likely an artifact of the phenomenon that the challenge in solar energy is to scale up, rather than down.

5. SIGNAL INTEGRATION AND TRANSDUCTION

All analytical systems have four basic components: a signal source that provides a stimulus to elicit a response; an input transducer in order to convert chemical information into an electrical signal; signal processing to modify the electrical signal to be of use; and an output transducer that converts the electrical signal into a display or command. In general, a sensor is a device that continuously and reversibly responds to the presence of a specific chemical species. Conversely a detector indicates a change in its surrounding environment. A biosensor is a self-contained integrated device which provides specific analytical information about biochemical mechanisms. Biosensors are typically packaged with a BRE that translates information from the biochemical domain into a chemical or physical output signal and a transducer that transfers the output signal into the electrical domain.[106] A critical component of any abiotic sensing system is the desire to incorporate BREs into a programmable sense and respond intelligent network. The central essence of this approach is to design and build a molecular electronic network that responds to a small perturbation with a signal cascade which, after some innate signal processing, causes the system to respond in an appropriate manner.[107] To be successful, it will require an effort to merge the BRE, signal transduction and processing into a single seamless step. Central to this effort will be the inclusion of research that aims to improve our understanding of how molecules transport charge; how biochemical systems transport charge across interfacial boundaries; and the networking BREs into nanoscale electronics for processing. Nanowire biosensors have already been demonstrated as a viable direct transduction element and could potentially serve as highly sophisticated interfaces between biological and nanoelectronic information processing systems.[108] Thus, there are three components: signal transduction, signal processing using molecular logic gates, and signal amplification and propagation.

6. CONCLUSIONS

The proliferation of the threat posed by biological and chemical agents, as well as the emergence of naturally occurring diseases, which pose a pandemic threat, argues against air "agent oriented" defensive posture, if only because the threat will not be known *à priori.* Therefore, a "target oriented" strategy is proposed, which defines the threat by its physiological effects. Because of the convergence of a number of technologies, it is now within reason to create completely artificial (i.e., abiotic) systems, which mimic the ability of living systems to sense, respond, and adapt to threats in the environment.

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